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Defining Protein Electrostatic Recognition Processes

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The objective of this proposal is to elucidate the nature of electrostatic forces controlling protein recognition processes by using a tightly coupled computational and interactive computer graphics approach. We developed the TURNIP program to determine the most favorable precollision orientations for two molecules by systematic search of all orientations and evaluation of the resulting electrostatic interactions. TURNIP was applied to the transient interaction between two electron transfer metalloproteins, plastocyanin and cytochrome c. Our results suggest that the productive electron-transfer complex involves interaction of the positive region of cytochrome c with the negative patch of plastocyanin, consistent with experimental data. Application of TURNIP to the formation of the stable complex between the HyHEL-5 antibody and its protein antigen lysozyme showed that long-distance electrostatic forces guide lysozyme toward the HyHEL-5 binding site, but do not fine tune its orientation. Determination of docked antigen/antibody complexes requires including steric as well as electrostatic interactions, as we have done for the U10 mutant of the anti-phosphorylcholine antibody S107. We have enhanced the graphics program Flex, a convenient desktop workstation program for visualizing molecular dynamics and normal mode motions. Flex now has a user interface and has been rewritten to use standard graphics libraries, so as to run on most desktop workstations.

electrostatic recognition, computer graphics, protein
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OBJECTIVE: To elucidate the nature of electrostatic forces controlling recognition processes.

APPROACH: Tightly coupled computational and interactive computer graphics programs were designed and used to predict and visualize electrostatic interactions of proteins. Results were compared with other experimental data.

ACCOMPLISHMENTS:

Development of TURNIP, a Program for Calculating Precollision Orientations.

The TURNIP program (Roberts et al., 1991) determines the most favorable precollision orientations for two molecules maintained at a specified distance by a systematic search of all orientations and evaluation of the resulting electrostatic interactions. A constant minimum separation distance between the two molecules was found to be essential for the algorithm to work robustly.

Precollision Orientations of an Electron-Transfer Complex. TURNIP was applied to the investigation of the transient interaction between two electron transfer metalloproteins, plastocyanin and cytochrome *c* (Roberts et al., 1991). The computational results were compared with the results found by visual alignment of electrostatic fields displayed with computer graphics. A computational search with TURNIP with a separation distance of 12 Å gave three energetically favorable families of orientations that positioned the the positive charges surrounding the heme edge on cytochrome *c* near the acidic patch surrounding Tyr 83 on plastocyanin. All dockings placed the heme edge of cytochrome *c* on the surface of plastocyanin and appeared to have no obstacles for further movement of cytochrome *c* towards the acidic patch of plastocyanin. None of the energetically favorable orientations placed the heme edge near His 87, the only exposed copper ligand of plastocyanin. In fact, dockings from the energetically favored precollision orientations indicated that movement of cytochrome *c* towards the His 87 region was sterically blocked by a solvent-exposed loop of conserved residues. Our results are consistent with the productive electron-transfer complex involving interaction of the positive region of cytochrome *c* with the negative patch of plastocyanin, which brings the heme edge into close proximity with Tyr 83, as indicated by experimental data. Thus, electrostatic forces appear to control the approach of these two electron-transfer partners during their transient interaction.

Investigation of Electrostatic Forces in Stable Binding Complexes. We have also completed our analysis of the U10 mutant of the

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phosphorylcholine-binding antibody S107, (Chien et al., 1989; Behar et al., 1989). This mutant antibody results from a single-site mutation of Asp 101 to Ala, over 9 Å distant from the antigen binding site, which results in a complete loss of phosphorylcholine binding activity. A combination of molecular dynamics and minimization techniques indicated that the mutant antibody may fold so that Arg 94 of the heavy chain extends into the antigen binding pocket to electrostatically and sterically block the binding of the hapten.

TURNIP was applied to the investigation of formation of the stable complex between the HyHEL-5 antibody and its protein antigen lysozyme. Computer graphics analysis of the electrostatic fields suggested that the association of these two molecules might be substantially aided by electrostatic forces. Unlike the transient electron-transfer interaction between plastocyanin and cytochrome *c*, however, the formation of this stable antibody-antigen complex appears to be only partially directed by electrostatic forces. Long-distance electrostatic forces guide lysozyme toward the HyHEL5 binding site, but do not fine tune its orientation.

Computer Graphics Program Development. We have also continued the development of the graphics program *Flex* (Pique et al., 1991), a convenient desktop workstation program, for the display of molecular dynamics and normal mode motions (Fisher et al., 1990). The program now has a user interface that allows multiple molecules to be displayed and manipulated separately, assisting the analysis of the multiple favorable orientations found by TURNIP. New features have been added, including main-chain ribbons and depth cueing, that increase the usefulness of the program (Pique et al., 1991). *Flex* has been rewritten to use the XView 2-D and PHIGS 3-D graphics libraries under the X-Windows protocol (developed at MIT), so that it runs on any machine with X, for example Silicon Graphics, Stardent, and Sun SPARC graphics workstations. The X-Windows protocol allows rapid communications between machines such that *Flex* can be run on other machines, such as our Cray YMP or Convex C2 supercomputers, and viewed on a desktop workstation.

We have also begun preliminary investigations of the graphics program AVS, which provides an environment for creating innovative customized applications, with the goal of converting our analysis programs into modules that would run inside the AVS environment. To explore the feasibility of this approach, we have written a template searching program that runs outside AVS but communicates with AVS to display results.

[illegible]

PUBLICATIONS:

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PATENTS:

None.

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